Brief Report Open Access

Iranian Journal of Diabetes and Obesity (IJDO)

The Effect of Interval Training on GCK Expression in Hepatocytes and Glucose Homeostasis in Type 2 Diabetes Rats

Fatemeh Nikseresht[1], Mostafa Bahrami[2*], Masoud Rahmati[3]

[1]PhD Student of Exercise Physiology, Sport Sciences Department, Lorestan University, Khorramabad, Iran.
[2]Assistant Professor of Exercise Physiology, Sport Sciences Department, Faculty of Literature and Human Science, Lorestan University, Khorramabad, Iran.
[3]Associated Professor of Exercise Physiology, Sport Sciences Department, Faculty of Literature and Human Science, Lorestan University, Khorramabad, Iran.

Abstract

Objective: Hepatic glucose release plays a potential role in hyperglycemia in type 2 diabetes (T2D) patients. The aim of this experimental study was to determine the effect of 6 weeks of high-intensity interval training (HIIT) on fasting levels of glucose and insulin as well as glucokinase (GCK) expression in liver tissue in obese T2D rats.

Materials and Methods: T2D was induced by a high-fat diet (HFD) and streptozotocin (STZ) intraperitoneal injection in 14 male wistar rats, then were randomly divided into HIIT (n=7) and control (n=7) groups. The HIIT group practiced 6-week HIIT (5 days/ weekly). Finally, 48 hours after the last session, fasting levels of glucose, insulin, and GCK expression in liver hepatocytes of both groups were measured and compared by independent T-test (SPSS, Version 22.0).

Results: HIIT resulted in a significant decrease of fasting glucose compared to the control group ($P<$ 0.0001). Compared with the control group, serum insulin (P: 0.018) and GCK expression in hepatocytes (P: 0.030) were significantly increased.

Conclusion: Based on these findings, the improvement in glucose in response to HIIT may be rooted in increased insulin and GCK expression in hepatocytes. However, understanding the mechanisms responsible for the effect of exercise training on the processes affecting hepatic glucose release requires further studies.

Keywords: Glucokinase expression, Hepatocyte, Interval training, Insulin

QR Code:

Citation: Nikseresht F, Bahrami M, Rahmati M. The Effect of Interval Training on GCK Expression in Hepatocytes and Glucose Homeostasis in Type 2 Diabetes Rats. IJDO. 2022; 14 (2) :105-109

URL: http://ijdo.ssu.ac.ir/article-1-707-en.html

10.18502/ijdo.v14i2.9454

Article info:

Received: 18 February 2022

Accepted: 08 April 2022

Published in May 2022

Corresponding Author:

Mostafa Bahrami, [2]Assistant Professor of Exercise Physiology, Sport Sciences Department, Faculty of Literature and Human Science, Lorestan University, Khorramabad, Iran.

Tel: (98) 916 661 2006

Email: mostafa_bahr2003@yahoo.com

Orcid ID: 0000-0002-2315-1894

Introduction

Clinical studies, especially in the last decade, have revealed that type 2 diabetes (T2D) is not only the result of insulin resistance or disruption of insulin signaling pathways in target tissue such as adipose tissue or muscle, but also defects in several mechanisms, prevalence or severity of the disease (1,2). On the other hand, the increase in blood glucose levels is also a result of the release of more than normal hepatic glucose.

In this context, in addition to gluconeogenic enzymes such as Fructose 1,6-diphosphatase (FDPase), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) that regulate the rate of G6Pase process (3), glucokinase (GCK) transcription disorder also severely affects hepatic glucose release which is primarily controlled and regulated by insulin (4). The main mediators of the effect of insulin on GCK are FOXO (Forkhead transcription factor family) transcription factors (5). The final pathway for glucose uptake and release, including glucose phosphorylation and dephosphorylation, is via GCK and G6Pase, respectively (5).

It seems that increased GCK activity or expression in hepatocytes, especially in T2Ds, is associated with a decrease in the severity of the disease and blood glucose in these patients. In this regard, Jacob et al. (2015) reported an increase in PEPCK and G6Pase expression in liver cells after one hour of running on a treadmill. They did not examine the expression of these transcription components after prolonged exercise (6). In the study by De Souza et al. (2010), a 2-hour swimming training session in the form of 4 steps (30 minutes for each step) reduced G6Pase expression and improved insulin signaling pathways in non-diabetic obese rats (7). However, the effect of exercise training especially interval training on GCK expression in hepatocytes is not seen. On the other hand, it has been pointed out that some optimal physiological adaptations resulting from long-term endurance training are achieved much faster in response to HIIT with less volume and training period (8). Therefore, considering the important role of GCK in the release of hepatic glucose and its dependence on insulin levels, the present study was aimed to determine the effect of HIIT on GCK expression in hepatocytes, blood glucose and serum insulin in T2D rats.

Materials and Methods

This experimental study consists of all male wistar rats of the Pasteur Institute of Iran, from which 14 rats 10-week-old (220± 20 g) were selected to participate in the study by accidentally. Based on other similar studies, the sample size was 7 rats in each group (9). After induction of T2D, diabetic rats were randomly divided into 7 groups of exercise (6 weeks of HIIT training) and control (no training). Rats were kept in a room under controlled light conditions (12:12 hours of light and darkness, with temperature (22± 2 °C) and 30-40% of humidity. They had free access to standard water and food. Throughout the research period, rats were moved by one person.

Induction of T2D

T2D was induced by a 6 weeks high-fat diet (HFD) and streptozotocin (STZ) intraperitoneal injection (30 mg/kg). To prepare HFD, 1% cholesterol powder and 1% pure corn oil were added to the standard rat diet (9). The HFD continued for both groups until the end of the study. One week after T2D induction, fasting blood glucose (FBS) was measured, and blood glucose between 150 and 400 mg / dL was considered as a ensure that mice developed T2D (10).

Training protocol

From the 18th week, the exercise group participated in an interval training for 6 weeks in 5 sessions of 27 minutes weekly in the form

of running on the treadmill with 40-second repetitions and 2-minute active rest between each repetition (9). 48 hours after the last training session, all rats were dissected (Table 1).

Sample Collection and Biochemical Assay

48 hours after the lasting exercise session, all fasted rats (10-12 hours overnight fast) were anesthetized by intraperitoneal injection of 10% ketamine (50 mg/kg) along with 2% xylosine (10 mg/kg). Blood samples were collected through cardiac puncture after anesthesia. Then, liver tissue was extracted and immersed in RNA later until biochemical analysis was performed to determine GCK expression. Blood samples were centrifuged for 10 minutes (3000 rpm) for serum separation. Glucose was determined by the glucose-oxidase method (Pars Azmoon kit, Tehran). Insulin was determined by ELISA method (Demeditec, Germany). Intra-assay and inter-assay coefficient of variation were 2.6% and 2.88 respectively.

RNA extraction/ Real-time PCR

To purify RNA, 20 milligrams of tissue were ground using a mortar and pestle, and extraction was then performed employing the RNeasy Protect Mini Kit (Qiagen, Germany) according to the manufacturer's guideline (15). The One Step SYBR Prime Script RT-PCR Kit (Takara Bio Inc, Japan) was employed according to the manufacturer's guideline to perform Real-time PCR. The thermal cycle program used for the Rotor-Gene Q instrument was as follows: 42˚C for 20 minutes, 95 ˚C for two minutes, and 40 cycles with 94˚C for 10 seconds and 60˚C for 40 second. RNA Polymrase II was used as a control gene. The sequence pattern of the primers is shown in Table 2.

Data analysis

All statistical analyzes were performed by SPSS / Win software version 22. Data were analyzed by independent T-test. Changes less than 5% were considered significant.

Ethical considerations

In this study, the protocol was approved by the Ethics Committee of Lorestan University (Ethics Code: LU.ECRA.2021/4).

Results

Body weight changes in both groups before and after exercise intervention are summarized in Table 3. No significant difference in body weight levels between the two groups was observed in the pre-study condition (*P*: 0.506). However, at the end of the study, bodyweight in the control group was significantly higher than in the exercise group (*P*< 0.0001). Also,

Table 1. Exercise protocol based on week and running speed in the active and rest phase in the exercise group

Weeks	Exercise Running speed (m / min)	Active rest walking speed (m / min)	Treadmill slope
1	25	10	5
2	25	10	10
3	28	10	10
4	32	10	10
5	35	10	10
6	35	10	10

* Running time in the exercise phase is 40 seconds and in the active rest phase is 2 minutes and the speed is in meters per minute.

Table 2. Primer sequence

Genes	Primer sequence	Product size	T m
GCK	**For**: AGGTGATGAGCCGGATGCAG **Rev**: CCCTCTCCCACTTTGACCAG	159 bp	60
RNA PolymraseII	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	164 bp	60

based on the findings of paired T-test, body weight at the end of the study did not change significantly compared to before HIIT exercises in the exercise group (*P*: 0.814) but increased significantly in the control group (*P*< 0.0001).

Data analysis by independent T-test indicates a significant difference in GCK expression in liver tissue between groups (*P*: 0.030). In other words, HIIT resulted in significant increase in GCK expression in the liver tissue of T2D rats (Table 4).

Compared to the control group, HIIT also led to a significant decrease in fasting glucose in the exercise group (*P*< 0.0001). Based on statistical findings, a significant increase was also observed in serum insulin in the exercise group in response to HIIT compared to the control group (*P*: 0.018) (Table 4).

Discussion

Increased GCK expression in liver tissue and decreased fasting glucose and serum insulin in the HIIT group compared to the control group, are the findings of the present study. Based on our knowledge, a few studies have been performed on GCK response to exercise. In the study of Cunha et al. the effects of 8 weeks of exercise at different intensities on GLUT4 and glycemic control in obese diabetic and non-diabetic rats showed that a high-intensity exercise program significantly improved blood glucose reduction compared to inactive individuals in the control group (11). It is also possible that, apart from the change in hepatic GCK expression in the present study, decreased blood glucose is rooted in increased serum insulin in response to HIIT exercise. Regardless of the effect of increasing insulin on gluconeogenic genes, increasing serum insulin levels in response to HIIT training improves glycemic profile by increasing glucose uptake by insulin-dependent muscle.

By binding to liver receptors, insulin regulates signaling cascades that activate enzymes involved in glucose uptake and release from the liver in the processes of gluconeogenesis, glycolysis, and glycogen metabolism. The presence of insulin in the liver stimulates GCK (12) and decreases G6Pase (13) expression, leading to long-term changes in GCK and G6Pase proteins that facilitate glucose release and uptake. Genetic regulation of GCK and G6Pase liver changes rapidly in response to changes in liver insulin in dogs (14), but changes in their protein levels have been observed to take several hours in response to changes in insulin in dogs (14). Although GCK measurement is one of the strengths of the present study, glucose improvement cannot be attributed to glucose changes alone. Because changes in glucose levels in response to exercise are dependent on other hormonal and genetic changes, the lack of measurement of other transcription factors affecting this process, such as PEPCK and G6Pase are of the limitations of the present study. Based on what was mentioned, the

Table 3. Body weight (g) in before and after training intervention in the study groups

Group	Pre-training	Post-training	* *P* (intra-group)
Control	280 (± 7)	275 (± 11)	0.612
Exercise	282 (± 10)	352 (± 2)	<0.0001
***P* (inter-group)	0.435	0.011	----

* inter-group represent comparison by independent T-test
** intra-group represent comparison by paired T-test

Table 4. Fasting glucose, serum insulin and GCK expression in liver tissue of exercise compared with control group (Mean ± SD).

Variable	Control group	Exercise group	*P**
Fasting glucose (mg/dl)	308 (± 17)	191 (± 19)	< 0.001
Serum insulin (µU/ml)	5.91 (± 0.35)	7.24 (± 1.23)	0.018
GCK expression	1	1.50 (± 0.54)	0.030

*independent T-test

decrease in blood glucose can be attributed to the decrease in hepatic glucose release. The change in GCK expression is probably rooted in the increase in insulin in response to HIIT, all of which provide the basis for reducing hepatic glucose release in these patients. Finally, it is noted that the improvement of glycemic profile in response to exercise cannot be attributed only to changes in liver, and understanding the mechanisms responsible for these changes in response to exercise requires more cellular-molecular studies.

Acknowledgments

We acknowledge the staff of the Pasteur Institute and the laboratory of Atieh Hospital for their genetic testing and ELISA.

Funding

This work was supported financially by Lorestan University, Iran.

Conflict of Interest

No conflict of interest was declared.

References

1. Wang J, Chen C, Wang RY. Influence of short-and long-term treadmill exercises on levels of ghrelin, obestatin and NPY in plasma and brain extraction of obese rats. Endocrine. 2008;33(1):77-83.
2. Mackelvie KJ, Meneilly GS, Elahi D, Wong AC, Barr SI, Chanoine JP. Regulation of appetite in lean and obese adolescents after exercise: role of acylated and desacyl ghrelin. The Journal of Clinical Endocrinology & Metabolism. 2007;92(2):648-54.
3. Wang Z, Dong C. Gluconeogenesis in cancer: function and regulation of PEPCK, FBPase, and G6Pase. Trends in cancer. 2019;5(1):30-45.
4. Zhang X, Yang S, Chen J, Su Z. Unraveling the regulation of hepatic gluconeogenesis. Frontiers in endocrinology. 2019:802.
5. Lin HV, Accili D. Hormonal regulation of hepatic glucose production in health and disease. Cell metabolism. 2011;14(1):9-19.
6. De Souza CT, Frederico MJ, Da Luz G, Cintra DE, Ropelle ER, Pauli JR, et al. Acute exercise reduces hepatic glucose production through inhibition of the Foxo1/HNF-4α pathway in insulin resistant mice. The Journal of physiology. 2010;588(12):2239-53.
7. Knudsen JG, Biensø RS, Hassing HA, Jakobsen AH, Pilegaard H. Exercise-induced regulation of key factors in substrate choice and gluconeogenesis in mouse liver. Molecular and cellular biochemistry. 2015;403(1):209-17.
8. Gibala MJ. High intensity interval training: new insights. Sports Science Exchange. 2007;20(2):1-8.
9. Karimi M, Eizadi M. The effect of interval training on FOXO1 expression in pancreas tissue of diabetes rats with high fat diet and STZ. Razi Journal of Medical Sciences. 2019;26(6):95-104.(in Persian)
10. McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, et al. Diagnosing insulin resistance in the general population. Diabetes care. 2001;24(3):460-4.
11. Cunha VN, de Paula Lima M, Motta-Santos D, Pesquero JL, de Andrade RV, de Almeida JA, et al. Role of exercise intensity on GLUT4 content, aerobic fitness and fasting plasma glucose in type 2 diabetic mice. Cell biochemistry and function. 2015;33(7):435-42.
12. Agius L. Glucokinase and molecular aspects of liver glycogen metabolism. Biochemical Journal. 2008;414(1):1-8.
13. O'brien RM, Streeper RS, Ayala JE, Stadelmaier BT, Hornbuckle LA. Insulin-regulated gene expression. Biochemical Society Transactions. 2001;29(4):552-8.
14. Ramnanan CJ, Edgerton DS, Rivera N, Irimia-Dominguez J, Farmer B, Neal DW, et al. Molecular characterization of insulin-mediated suppression of hepatic glucose production in vivo. Diabetes. 2010;59(6):1302-11.